

potassium (BK_{Ca}) channels and inducing membrane hyperpolarization and vasorelaxation.

In Fluo-4-AM-loaded mesenteric myocytes, application of the Epac-specific cAMP analogue 8-pCPT-2'-O-Me-cAMP-AM (10 μ M) increased spark frequency from 0.045 ± 0.008 sparks/s/ μ m under basal conditions to 0.103 ± 0.022 sparks/s/ μ m ($p < 0.05$). Importantly this increase also occurred in the presence of myristoylated PKI amide (14-22), a potent and selective inhibitor of PKA.

Application of 8-pCPT-2'-O-Me-cAMP-AM (5 μ M) reversibly increased both the frequency (0.94 ± 0.25 to 2.30 ± 0.72 s⁻¹) and amplitude (23.9 ± 3.3 to 35.8 ± 7.7 pA) of spontaneous transient outward currents (STOCs) recorded in isolated mesenteric myocytes ($n=7$; $p < 0.05$). These currents were sensitive to the selective BK_{Ca} channel blocker, iberiotoxin (100 nM), and to ryanodine (30 μ M). In addition, current clamp recordings of isolated myocytes showed a 7.43 ± 0.96 mV ($n=4$) hyperpolarization in response to exposure to 8-pCPT-2'-O-Me-cAMP-AM (5 μ M).

Our data suggest a novel cAMP-dependent mechanism in mesenteric smooth muscle cells whereby activation of Epac facilitates localized Ca²⁺ release which activates surface BK_{Ca} channels to modulate membrane potential and vascular tone.

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2. Bos, JL (2006). Trends in Biol. Sci. 31:680-686

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Methods to Measure and Analyze Ciliary Beat Activity: Calcium Influx-Mediated Cilia Mechanosensitivity

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Airway ciliary beat activity (CBA) plays a pivotal role in protecting the body by removing mucus and pathogens from the respiratory tract. Since CBA is complicated and can not be characterized by merely frequency, we recorded CBA using laser confocal line scanning and defined 6 parameters for describing CBA. The values of these parameters were all above 0 when measured in beating ciliated cells from mouse tracheae. We subsequently used 10 μ M adenosine-5'-triphosphate (ATP) to stimulate ciliated cells and simultaneously recorded intracellular Ca²⁺ levels and CBA. We found that intracellular Ca²⁺ levels first increased, followed by an increase in CBA. Among the 6 parameters, frequency, amplitude, and integrated area significantly increased, whereas rise time, decay time, and full duration at half maximum markedly decreased. The results suggest that these 6 parameters are appropriate for assessing CBA and that increased intracellular Ca²⁺ levels might enhance CBA. We next used our established methods to observe changes in mechanically stimulated cilia tips. We found that mechanical stimulation-induced changes in both intracellular Ca²⁺ levels and CBA were not only similar to those induced by ATP but were also blocked by treatment with a Ca²⁺ chelator, BAPTA-AM (10 μ M) for 10 min. Moreover, while the same blockage were observed under Ca²⁺-free conditions, addition of 2 mM Ca²⁺ into the chamber restored increases in both intracellular Ca²⁺ levels and CBA. Taken together, we have provided a novel method for real time measurement and complete analysis of CBA as well as demonstrated that mechanical stimulation of cilia tips resulted in Ca²⁺ influx that led to increased intracellular Ca²⁺ levels, which in turn triggered CBA enhancement.

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Spatial Distribution Patterns of Alpha-CaMKII in Hippocampal Tissue Suggest Role for Transient Not Persistent Translocation in Chemical LTP and Persistent Clustering in Ischemia

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Calcium/calmodulin-dependent protein kinase II (alpha-CaMKII) forms punctate structures in dendritic spines and in the soma of hippocampal pyramidal neurons upon calcium elevation evoked by N-methyl-D-aspartic acid receptor (NMDAR) stimulation. Though advocated to be relevant to memory formation, it is not clear whether spinal accumulation represents translocation to and interaction with NMDAR or, as somal punctate accumulation, it is the result of self-association representing clustering of alpha-CaMKII. Alpha-CaMKII punctate accumulation can be transient or persistent, the significance of temporal pattern to memory formation and excitotoxicity is however not clear either. In order to

obtain a clearer understanding of the state of alpha-CaMKII in memory formation and excitotoxic insult, e.g. ischemia, we carried out an extensive ultrastructural study of alpha-CaMKII distribution in control, in chemically induced long-term potentiation (cLTP) and ischemic conditions in rat CA1 hippocampal slices by electron microscopy and immunogold labeling. We found preferential localization of alpha-CaMKII to post-synaptic densities in all three conditions but no significant differences between the distributions of alpha-CaMKII, phospho-Thr286-alpha-CaMKII and phospho-Thr305-alpha-CaMKII when comparing control and cLTP treated slices. The number of alpha-CaMKII clusters was significantly greater in the ischemic than in the control and cLTP treated slices. Thus, contrary to expectations, we found no evidence of significant persistent alpha-CaMKII accumulation in the dendritic spines in cLTP and conclude that transient rather than persistent translocation of alpha-CaMKII to the post-synaptic membrane is relevant to LTP induction. This is consistent with a switch-like rather than a resident role for alpha-CaMKII in memory formation. Our data further show that cluster formation is a hallmark of ischemic insult and not relevant to memory formation.

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Orai-Stim Mediated Ca²⁺ Release from Secretory Granules Revealed by a Novel Ca²⁺ and pH Probe

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Secretory granules (SGs) bud from the trans-Golgi network and during subsequent maturation, acquire an acidic intraluminal pH, electron-dense cores, and matrices that bind Ca²⁺ and other ions. Total Ca²⁺ in SGs is remarkably high, 30-40 mM. The free Ca²⁺ is significantly lower, with estimates ranging from 10-80 μ M. Understanding roles for this calcium and potential mechanisms of release is hampered by the difficulty in measuring SG calcium directly in living cells. We adapted the FRET-based D1-ER probe to develop a novel probe (D1-SG) to measure calcium and pH in secretory granules. It significantly localizes to SGs and reports resting free Ca²⁺ of 69 ± 15 μ M and pH 5.8. Application of ATP resulted in a slow monotonic decrease in SG Ca²⁺ temporally correlated with the occurrence of store-operated calcium-entry (SOCE). Further investigation revealed a novel receptor-mediated mechanism of calcium release from SGs that involves store-operated channels. SG Ca²⁺ release is completely antagonized by a SOCE antagonist, by switching to Ca²⁺-free medium, and by overexpression of a dominant-negative Orai1(E106A). Overexpression of the CRAC activation domain (CAD) of STIM1 resulted in a decrease of resting SG Ca²⁺ by ~75% and completely abolished the ATP-mediated release of Ca²⁺ from SG's. Overexpression of a dominant negative CAD construct (CAD-A376K) induced no significant changes in SG Ca²⁺. Colocalization analysis suggests that, like the plasma membrane, SG membranes also possess Orai1 channels, and during SG Ca²⁺ release, colocalization between SGs and STIM1 increases. We propose Orai channel opening on SGs membranes as a potential new mode of calcium release from SGs that may serve to raise local cytoplasmic calcium concentrations and aid in refilling intracellular calcium stores of the endoplasmic reticulum and exocytosis. (Grants NS08174/GM83913/F32 DC068982/K01 MH086119/NSFCChina/985DEChina).

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Structural Requirements of N- and C-Terminal Orai Strands to allow Maximal Store-Operated Ca²⁺ Current Activation by STIM1

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The Ca²⁺ sensor protein STIM1 anchored in the ER membrane and the pore-forming Orai protein in the plasma membrane represent the key components of Ca²⁺ release-activated Ca²⁺ (CRAC) channels. Recent publications have demonstrated that CRAC current activation requires 8 STIM1 molecules together with an Orai complex formed by 4 subunits. Channel gating necessitates direct binding of the cytosolic portion of STIM1 with both N- and C-termini of the Orai channel. A single point mutation L273S in Orai1 C-terminus abolishes the interaction with STIM1 and followingly the activation of Orai1 currents. A truncation till to the fourth amino acid of the N-terminal conserved region (aa73-89) results in the loss of STIM1 binding to the N-terminus and STIM1-mediated activation. We employed Orai1 dimers to identify whether all N-/C-terminal domains within an Orai concatamer are required to be intact for maximal Ca²⁺ current activation. Orai dimers with one wild-type and one L273S-mutant subunit displayed a similar extent of maximal currents like and an unchanged inactivation compared to wild-type proteins. These results suggest that either less than 8 STIM1 are even sufficient to activate Orai1 channels maximally or the L273S mutation is not sufficient to impair the recruitment of eight STIM1 proteins to an Orai1 oligomer containing